CARBONIC ANHYDRASE AND GROWTH IN THE OYSTER AND BUSYCON [1, 2]

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The enzyme carbonic anhydrase is present in the shell-forming mantle tissue of a large number of mollusks (van Goor, 1948; Freeman and Wilbur, 1948). During the course of an investigation of the possible importance of this enzyme in the deposition of carbonate in shell formation it was noted that the carbonic anhydrase concentration of the conch *Busycon carica* was considerably less in larvae just prior to shell formation than in those which had recently acquired a shell. This suggested that the enzyme content may increase with development and raised the question as to the constancy of the enzyme content of the mantle tissue with growth of the adult. These aspects of carbonic anhydrase activity have been examined in the present study. The alteration of enzyme activity during growth has been investigated on the whole larvae of *Busycon carica*. For a study of enzyme activity during growth of adult mantle the oyster *Ostrea virginica* has been used. The results indicate that the carbonic anhydrase activity varies in a regular manner in these forms both during development and with growth of the mantle of the adult.

Methods

Ostrea mantle tissue and whole Busycon larvae taken from egg cases were ground and usually frozen. The tissue was then extracted in 3 to 9 times its weight of distilled water for two or more hours. Oyster sperm and eggs were washed in sea water by centrifugation before extraction. Tissue samples to be compared for enzyme activity were prepared at the same time and received identical treatment. The extracted material was centrifuged briefly to remove tissue and shell fragments and the carbonic anhydrase activity of the supernate was measured colorimetrically using a veronal buffer (Wilbur and Anderson, 1948). Sulfanilamide, a powerful inhibitor of mammalian carbonic anhydrase (Mann and Keilin, 1940), was found to inhibit the enzyme in Busycon and Ostrea. Accordingly, an aliquot of an experimental sample to which sufficient sulfanilamide was added served as a very satisfactory control. By comparing the rate of enzyme action in the presence and absence of sulfanilamide the action of tissue substances which affect the pH and buffer capacity of the medium is cancelled. The enzyme activity was calculated

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from rates of the catalyzed and uncatalyzed reaction and expressed in units following the method of Main and Locke (1941).

Unfortunately no single tissue unit will serve satisfactorily as a basis for comparison for all the tissues employed. For mantle tissue, wet weight and dry weight have been used. For whole Busycon larvae, both with and without shell, the enzyme activity is expressed as units per individual and also per unit volume as determined by centrifuging thoroughly macerated larvae at ca. 825 × g. for 5 minutes. The latter method was also used for Ostrea eggs and sperm.

Results

Busycon larvae

The eggs of Busycon are laid in a string of flattened, disk-like cases which commonly contain two to three dozen or more individuals. Since the formation of the string of cases continues for some period, one has available for study in a single

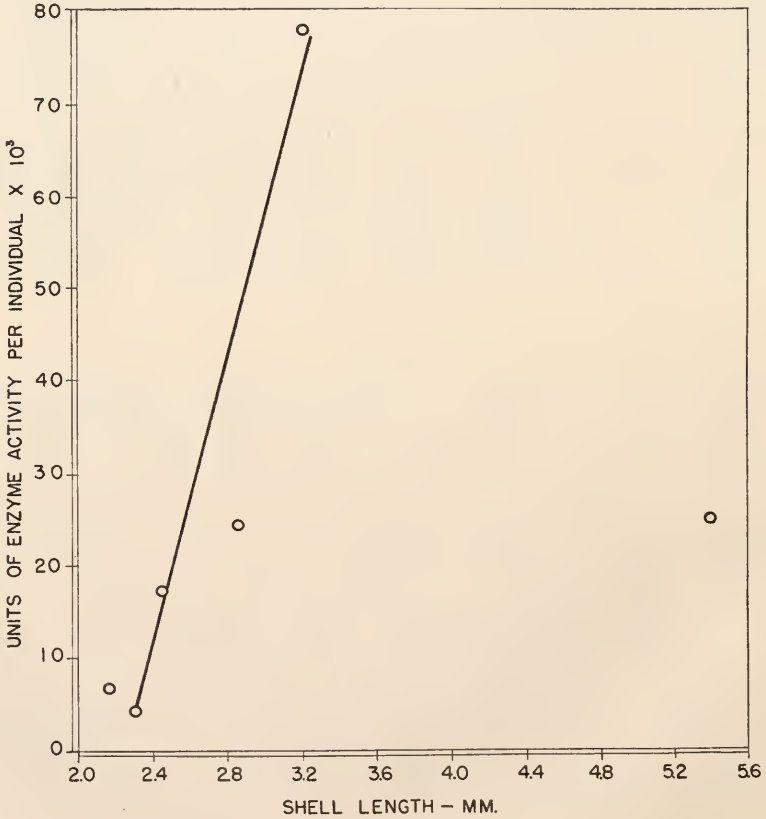

Figure 1. Carbonic anhydrase activity of Busycon larvae. Approximately 300 larvae of similar size from a single egg string were extracted for the determination of each point. Several egg strings were used. A similar relationship is obtained on plotting enzyme activity vs. unit volume.

string a series of developmental stages both with and without shell. The carbonic anhydrase activity of naked larvae and those with shell from such a string was measured on a sample of about 300 individuals of each type. The enzyme activity per individual was found to be 0.0049 units for those without shell and 0.23 units for those with shell, or an increase of 47 fold. The jelly within the egg cases was without enzyme activity.

The difference in enzyme activity in the naked and shelled stages suggested an examination of possible variation in enzyme activity with development in a series in which all larvae were depositing shell. Lots of 150 to 300 individuals in a desired size range were collected. A sample of 16 to 32 individuals was taken for an estimate of the average length, and the entire lot was then used for the measurement of enzyme activity. When the enzyme activity per individual is plotted against average length (Fig. 1) it is seen that the activity increases very markedly during the early stages of shell deposition. The activity of an individual 3.2 mm. in length may be nearly 20 times that of one 2.2 mm. in length. Clearly the increase per unit of tissue is also very great indeed. This may be shown by expressing the activity in terms of unit volume as determined by centrifuging macerated larvae, even though a large fraction of the total is shell. Whether enzyme activity falls off for individuals larger than 3.2 mm. (Fig. 1) or whether the value for the 5.4 mm. stage is aberrant is not known.

The changes in carbonic anhydrase activity with growth in Busycon reported here represent the enzyme activity of the whole larva. If the increase in enzyme activity found in Busycon is to be correlated specifically with shell deposition, the mantle tissue should also show the increase, since this tissue is responsible for shell formation. Unfortunately the measurement of enzyme activity of the mantle of individuals of 2 to 6 mm. in length will have to await the development of suitable micro methods.[4] The relation between size and enzyme activity of the adult mantle has, however, been investigated in Busycon and also in Ostrea.

Adult Busycon

Individuals ranging in length from 5 to 20 cm. and weighing from 8.9 to 587 gms. were used. Because of the difficulty of grinding mantle tissue, pieces of mantle were sliced very thin with a razor while frozen and extracted at low temperature for 48 hours. In a single series examined no consistent relationship between size and enzyme activity was found, although there was some slight suggestion that the mantles of the largest individuals had a somewhat lower enzyme content than the smallest individuals on a wet weight basis. Further study on this species would be desirable.

Adult Ostrea

In the adult oyster a definite relation was found between size and enzyme activity. The activity per unit dry weight or wet weight of mantle was in general inversely proportional to the length of the shell (Fig. 2). The activity of the smallest individuals may be several times that of the largest in any one collection.

The curves for oysters from two beds a few hundred yards apart are character-

[4] A micro method for carbonic anhydrase is being devised for such quantities of tissue.

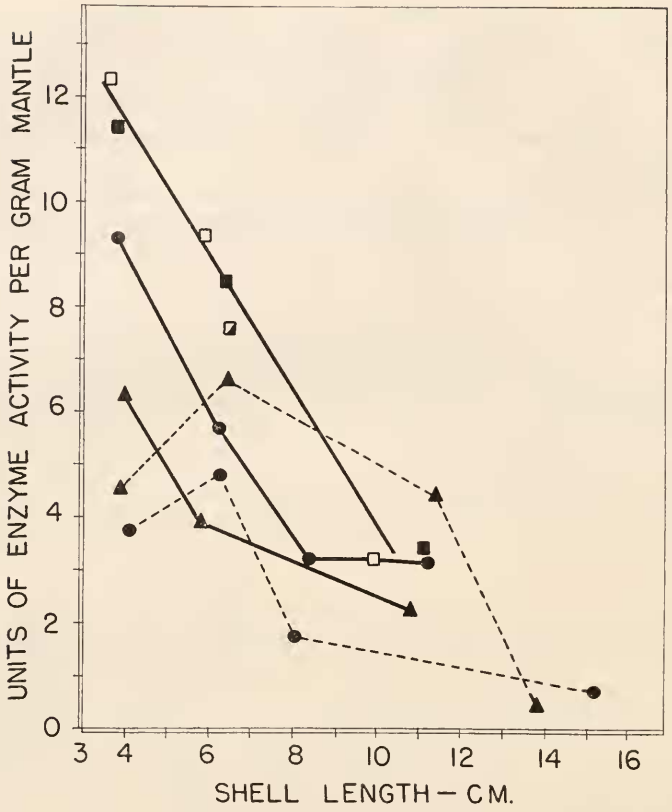

FIGURE 2. Carbonic anhydrase activity of oyster mantle. Enzyme activity is expressed per gm. wet weight. Dry weight gives a similar relationship. The shell length represents average measurements on the shorter shell. Data plotted with solid lines (————) are for oysters taken from a bed located on a sandy beach exposed to relatively rapid water movement. Data plotted with dashed lines (- - - -) are for oysters taken from an intertidal bed in muck containing fuel oil from ships and in a location not exposed to fast currents.

□ July 12, 1948.
■ July 12, 1948. Oysters kept in air 6½–7½ hours.
◪ July 12, 1948. Oysters kept in air 6½–7½ hours, then returned to water for 13 hours.
▲ October 31, 1948.
● March 5–6, 1949.

istically distinct both in their form and with respect to seasonal differences.[5] The data indicated in solid lines in Figure 2 were obtained from oysters collected between low and high tide levels from a sand beach exposed to relatively rapid water movement. Dashed curves (Fig. 2) are for oysters growing between tide levels in muck containing fuel oil from ships and in a location which was not subjected to fast currents.

[5] Dr. A. F. Chestnut of the Institute of Fisheries Research of the University of North Carolina, Morehead City, N. C., found active deposition of shell by oysters in this region throughout this period.

The enzyme activity of oysters exposed to air for 6½ to 7½ hours (Fig. 2, ■) was the same as that for oysters taken directly from sea water (Fig. 2, □).

By extrapolation of the curves in Figure 2 one might predict the size of oyster which would be completely deficient in carbonic anhydrase. Interestingly enough, oysters of this size have not been found in the beds from which the experimental material was collected.

Discussion

The presence of carbonic anhydrase in a wide variety of tissues of animals belonging to several phyla suggests that the enzyme plays a general role in the handling of metabolic carbon dioxide (van Goor, 1948). Such a role seems likely in mollusks as well, for the enzyme is present in several tissues. An analysis of washed eggs and sperm of the oyster showed that both gametes also contain carbonic anhydrase. The eggs had an activity of 3.6 units and the sperm 3.4 units per ml. of packed cells. Here too a general rather than a specific function appears probable. With differentiation the concentration of the enzyme may increase as described for Busycon larvae and has also been found in vertebrate tissues (Van Goor, 1948). Whether the marked increase in carbonic anhydrase bears a general relation to development and differentiation or has a more specific function in the rapid deposition of shell which occurs at this time, is not known.

In contrast to Busycon larvae, enzyme activity of the adult oyster mantle was found to decrease with increasing size. The possible physiological significance of this relationship for shell deposition obviously depends upon whether the enzyme activity becomes a limiting factor in the processes concerned with shell formation. If the enzyme is in excess, as it is in mammalian erythrocytes, a reduction may be of no consequence. An evaluation of the part played by carbonic anhydrase is being approached by studying the influence of enzyme inhibitors on the rate of shell growth as indicated by the deposition of radioisotopes.

The change in carbonic anhydrase content of the oyster mantle with age does not in itself reflect a decreased ability to synthesize the enzyme but may be due to a relative increase in cells or material not directly involved with carbonic anhydrase. Speculation in this regard appears unprofitable until such time as a histochemical technique for the enzyme can be applied to the mantle tissue.

The variation of carbonic anhydrase activity of the oyster mantle with size, habitat, and with the season suggests that these factors should be considered in comparing carbonic anhydrase activity of species of mollusks and perhaps other invertebrates.

Summary

1. The carbonic anhydrase activity of *Busycon carica* was found to increase markedly during development in the egg case. The enzyme activity of whole larvae during the early stages of shell formation may be more than 40 times that of larvae just prior to shell deposition. Larvae with shell may show an increase of nearly 20 fold in enzyme activity in growing from a length of 2.2 mm. to 3.2 mm.

2. In the mantle of the adult oyster *Ostrea virginica* the carbonic anhydrase activity was in general inversely proportional to the length of the shell. The activity per unit weight of mantle of small oysters may be several times that of large

oysters growing in the same location. Curves relating enzyme activity of the mantle to the size of the oyster were distinctly different for oysters growing in different habitats.

3. Carbonic anhydrase is present in both gametes of the oyster, the concentration per unit volume being of the same order in both.

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